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## PLASMA PROTEINS AND WOUND HEALING

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INJURY elicits a number of metabolic and physiologic responses at the site of tissue damage and throughout the body. Among these changes are the increased hepatic synthesis of a number of plasma proteins, the acute phase response, which results in an increase in the plasma concentration of these proteins (26, 98). Some of these proteins appear to localize specifically around the site of injury (2, 34) while others accumulate at the wound seemingly as a result of greater capillary permeability (7). There is considerable evidence, in some instances admittedly circumstantial, that the resultant increase in plasma protein availability markedly facilitates wound healing. This review was undertaken to assemble and evaluate this evidence.

## DISCUSSION

Even in normal individuals, plasma proteins which constitute only 3.5 per cent of total body protein, represent 10 per cent of whole body protein turnover (96) and may act as an important form of amino acid transport from the liver to peripheral tissues (21). Albumin, the major plasma protein, appears to be an amino acid donor for extrahepatic tissue synthesis in growing rats (42). Decreased total plasma protein or albumin concentrations seem to be associated with reduced wound tensile strength in a variety of clinical states: protein depleted diet, wound infection, uremia and old age (68). Edematous swelling which is caused by hypoproteinemia is apparently not responsible for this impairment in healing

(13). Sixty-five to 70 per cent of albumin catabolism normally takes place in peripheral tissues (97). The rate of this catabolism increases as a result of injury (7, 67), as does the turnover rate of several of the other plasma proteins (23, 67, 103). Extravasation of albumin appears to be regulated, in part, by localized mediators through the adenosine 3':5'-cyclic phosphate system (44). Thus to some degree, albumin is made available to regenerating tissue in proportion to the extent of injury and inflammation. Although albumin is not degraded in its native conformation by macrophages (46), such changes in temperature and pH which might occur at the site of inflammation are sufficient to denature albumin (95). This would make it susceptible to degradation and allow use of its components by macrophages participating in wound repair (53, 87).

Albumin also acts as an amino acid transport agent by binding significant amounts of amino acids such as tryptophan and cysteine. Tryptophan has been shown to be capable, under certain conditions, of regulating and maintaining hepatic protein synthesis through polysome stabilization (81, 101). However, in muscle, leucine and the branched chain amino acids are considered to have a greater effect on protein synthesis than tryptophan (9). In view of the possible restricted availability of other amino acids at the wound site, tryptophan may still exert a regulating action. The sulphur amino acids, cysteine in particular, are known to stimulate collagen synthesis (100). Both of these amino acids have a low residue content intrinsic to human albumin and if bound to albumin, may provide a more balanced nutritional source than albumin alone.

In addition to amino acids, albumin also binds zinc and fatty acids. Zinc plays a role in collagen cross-linking (56). Zinc deficiency results in ab-

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normal keratinization and reduced wound tensile strength (20) and alters glycine incorporation into collagen (88). Zinc deficiency also results in reduced methionine incorporation into plasma, liver, kidney and muscle protein (38). Zinc supplementation, particularly in patients with zinc depletion, results in improved wound healing (31). Free fatty acids which are primarily transported in blood by albumin provide an energy source other than amino acids or carbohydrates as well as precursors for membrane synthesis. Albumin accumulation at a wound site may help to provide zinc and energy substrates for wound healing.

Though albumin may play a significant role in peripheral nitrogen metabolism in normal individuals and wound nutrition during minor to moderate injury, it does not appear to be an essential element of wound healing or tissue development, for there have been instances of analbuminemia in patients (94) and rats (64) without reports of obvious impairment in growth, wound healing, or both. In addition, during severe injury albumin synthesis appears to decline; thus, albumin is likely to play less of a nutritive role under such circumstances.

Acute phase protein concentration and synthesis increase during injury (23, 26, 67, 98, 103) and, thus, acute phase proteins are more likely to play a major role in wound healing. In rats with turpentine abscesses, infused  $^{14}\text{C}$ -hexosamine rapidly appears in the carbohydrate moiety of acute phase proteins and is subsequently found as part of granulation tissue (41). Radiolabeled amino acids from hemoglobin (19) and haptoglobin (40) are also apparently used in tissue repair; the contribution of amino acids from these two proteins may depend upon the extent of hemolysis.

Protease inhibitors also accumulate at the site of injury, presumably to prevent additional damage due to proteolytic enzymes released from already damaged tissue as well as from phagocytic cells. The principal protease inhibitors in man are  $\alpha_1$ -antitrypsin and  $\alpha_2$ -macroglobulin. Once bound to  $\alpha_1$ -antitrypsin, proteases are completely inhibited, while those bound to  $\alpha_2$ -macroglobulin retain some activity. There appears to be a transfer of proteases from  $\alpha_1$ -antitrypsin to  $\alpha_2$ -macroglobulin (66) and a highly preferential and rapid uptake of  $\alpha_2$ -macroglobulin-protease complexes, but not  $\alpha_1$ -antitrypsin-protease complexes, by macrophages (15, 16, 17). Fibroblasts also clear  $\alpha_2$ -macroglobulin-protease complexes (90). Since

$\alpha_2$ -macroglobulin is the major circulating zinc glycoprotein, containing approximately 40 per cent of the total serum zinc concentration, localized degradation of  $\alpha_2$ -macroglobulin-protease complexes at the wound site could provide zinc, amino acids and carbohydrate in addition to regulating the local concentration of proteases.

Similar to  $\alpha_2$ -macroglobulin,  $\alpha_1$ -acid glycoprotein, orosomucoid, accumulates at the site of infarct after myocardial infarction (2). Turnover of this protein has been shown to increase after injury, such as thermal burns (104) or sterile abscesses (103). Of the plasma proteins,  $\alpha_1$ -acid glycoprotein has one of the highest carbohydrate contents, 41 to 45 per cent and in addition has structural homologic characteristics to both the heavy and light chains of IgG (82). As a result of its structural properties, this glycoprotein may act as an adhesive agent in wound healing. At the same time, orosomucoid may afford protection to healing tissue against autoimmune reactions, since phagocytosis (91) and lymphocyte transformation (14) are inhibited by  $\alpha_1$ -acid glycoprotein.  $\alpha_1$ -acid glycoprotein has also been shown to affect the spacing of collagen fibers formed from soluble collagen (27) and to inhibit adenosine diphosphate and adrenaline induced platelet aggregation (84). The end result of these actions may be to localize clot formation specifically at the site of wound repair, masking normally hidden but potentially antigenic loci and preventing the production of autoantibodies. In addition, orosomucoid has been shown to bind a number of cationic drugs, such as the catecholamine analogue, alprenolol hydrochloride (70) and steroid hormones, such as progesterone (28), reducing their activity.

Ceruloplasmin, another plasma glycoprotein which increases rapidly following injury and inflammation, is the principal copper metal protein in blood. Eighty to 95 per cent of total circulating copper is bound to ceruloplasmin, the rest being more loosely associated with albumin and amino acids (72). Although there is some controversy as to whether or not copper is delivered to tissues primarily bound to albumin or to ceruloplasmin, ceruloplasmin appears to be the primary copper transport protein for transferring copper to cytochrome C oxidase (37, 72), vital to aerobic energy production, which along with glycolysis (39) increases during wound healing (25). Ceruloplasmin and the copper it carries are essential to collagen formation, and, both ascorbate (12) and cysteine (3), used for

wound healing in their oxidized form, are oxidized by ceruloplasmin. Copper is essential for lysyl oxidase and the extracellular cross linking and maturation of collagen and elastin (65). Ceruloplasmin and the copper it contains may also act to protect the matrix of healing tissue against superoxide ions, generated by phagocytes in the course of clearing tissue debris or microorganisms, which foster the degradation of hyaluronic acid. A correlation has been found between the antioxidant activity of serum from rheumatoid arthritic patients and ceruloplasmin, which is hypothesized to be related to an increase in superoxide dismutase mimetic activity (85). In addition, ceruloplasmin supplies copper to the tissue superoxide dismutase, cytochrome c (54). Ceruloplasmin deficiency causes a decrease in phospholipid synthesis necessary for cell membrane production (1) in regenerating tissue.

An elevation of C-reactive protein in serum or other serous fluids, such as wound exudates, is generally considered to be a nonspecific indicator of any process resulting in tissue necrosis and inflammation (10). C-reactive protein also is reported to localize at the site of injury following myocardial infarction or local inflammation (50, 51). It has been hypothesized that C-reactive protein is partially responsible for postoperative anergy because, although it is not cytotoxic, it does inhibit the proliferation of certain T-cells (61) and lymphokine production (60). There is some evidence that this acute phase protein, which binds to 30 to 40 per cent of all peripheral lymphocytes (11), preferentially binds to T-cell derived lymphocytes which have IgG Fc receptors (99). Since these cells, Tg cells, have suppressor activity for B-cells in vitro (58), C-reactive protein may function as a stimulator of immunoglobulin production at the expense of some cell-mediated immunity. If this is so, cell-mediated immunity should be depressed, but immunoglobulin synthesis enhanced following injury. Mild to moderate operative and thermal injuries produce just such a response; decreased cell-mediated immunity (36) and enhanced generation of cells which form antibodies, seemingly in proportion to the extent of injury (45, 74). Severe thermal injury results in a depression in both T-lymphocyte and B-lymphocyte populations (92), yet even so there is a relative increase in B-cells within the first week postburn (52). C-reactive protein may also facilitate tissue repair by stimulating opsonization of microorganisms (29) and, thus, reduce wound infection. This protein also inhibits platelet aggregation activat-

ed by thrombin and subsequent release of lysosomal enzymes such as  $\beta$ -glucuronidase (24), thus regulating clotting and preventing the release of potentially destructive cellular enzymes.

Haptoglobin increases four to five times its normal plasma concentration following injury and is frequently found in inflammatory exudates (73). The principal biologic function of this protein appears to be the removal of free hemoglobin released by hemolysis (40). Hemoglobin irreversibly binds to haptoglobin, forming a complex which is rapidly cleared by the reticuloendothelial system (69). Hemoglobin-bound haptoglobin has a half-life of 90 minutes in contrast to three days for free haptoglobin (22). This complex has a peroxidase activity not characteristic of haptoglobin per se (73), which may be related to its reported stimulation of proline incorporation into collagen (6). Iron is one of the cofactors of proline hydroxylase, so the complex, if catabolized at the site of injury, could act as an iron donor for proline hydroxylase synthesis. Haptoglobin also may prevent retardation of wound healing caused by infection by reducing the availability of iron to invasive organisms (8). A similar function has been ascribed to transferrin, the major iron transport protein in the absence of injury and to lactoferrin (8). Since anhaptoalbuminemics appear to be otherwise healthy (73), an essential role for haptoglobin in the reparative processes is doubtful.

Fibrinogen accumulates at the site of injury for the first or second week after operation (34) and, in the presence of enzymes released from blood and cells in the surrounding tissues, forms fibrin. Fibrin increases the tensile strength of the wound and stimulates fibroblast proliferation and growth (71). Fibrin has been used clinically as a tissue adhesive (86) and promotes healing of soft tissue (5) and cartilage after osteotomy, as well as preventing suture leakage and reducing mortality after abdominal operations (93). Fibrin is not only essential for hemostasis, but may provide a mechanical barrier to invasive organisms. However, there is a report indicating that fibrin trapping of microorganisms might be involved in the pathogenesis of bacterial endocarditis (18).

Fibrin-stabilizing factor, Factor XIII, which converts soluble noncovalently linked fibrin to an insoluble covalently bound form, has been shown to speed wound (49) and fracture healing (4) and to increase breaking strength. Circulating levels of Factor XIII fall postoperatively. A persistent depression of plasma Factor XIII is as-

sociated with wound dehiscence. After normal healing, the concentration rises significantly (83).

Cold insoluble globulin, probably identical to fibronectin (75) and alpha<sub>2</sub>-SB-glycoprotein (79) is also covalently bonded by Factor XIII to fibrin (62). This protein has previously been shown to concentrate preferentially at the site of wound repair, particularly where inflammation is greatest and fibrous tissue most dense (57). It does not increase the breaking strength of the wound (86), but does appear to have an important role in platelet spreading and lysis on a protein matrix (30) though not adhesion (80). Fibronectin also binds to all mammalian collagens that have been tested to date (35), and mediates cell attachment to collagen-coated surfaces (48). This protein has been shown to be synthesized by a wide variety of cells including macrophages (43) and fibroblasts (76). It is also released from platelets treated with collagen or thrombin (105) and is found as a cell surface protein on T-cell but not on B-cell derived lymphocytes (33). Acting as an essential glue for most of these cellular components of wound healing, increasing adhesion, cellular mobility and cell to cell social interactions within the developing fibrin-collagen matrix of regenerating tissues (102), fibronectin has a potentially major role in wound healing. As an additional function, fibronectin or alpha<sub>2</sub>-SB-glycoprotein has also been shown to stimulate opsonization (77) and to be essential to reticulo-endothelial system activity. The circulating concentration of this protein is frequently low after surgical trauma (55). A cryoprecipitate fraction of serum rich in fibronectin has recently been infused into surgical patients, diminishing septicemia, pulmonary insufficiency and the length of recovery period (78).

Although it was postulated that plasma proteins, in particular the acute phase globulins, facilitate wound healing in a variety of ways, it is conceivable that the excessive quantities of these potent proteins that are produced following massive injury, such as burns of more than 50 per cent of the total body surface, may also have deleterious effects. For example, an excess of alpha<sub>1</sub>-antitrypsin might so suppress superoxide production by leukocytes (47) and lymphocyte blastogenesis (89) as to compromise killing of microorganisms and development of immunity. Alpha<sub>2</sub>-macroglobulin-protease complexes may be generated in sufficient quantities and duration not only to produce the transient anergy which may protect against subsequent autoimmune dis-

ease (63), but to preclude efficient killing and clearance of microorganisms. C-reactive protein which also may be involved in promoting the transitory potentially beneficial immunosuppression could achieve and persist at such concentrations so as to suppress B-cell as well as T-cell function (59), again compromising host resistance to infection.

From this limited summary of the possible functions of individual plasma proteins, it can be seen that acting in concert they may have a major role in the support of the cellular activities essential to the repair of tissue injury in man. The importance of plasma protein modulation of hormonal action is more conjectural. In normal man, some of these proteins, like albumin, are important in the transport of hormones. At the site of regenerating tissue, many of these plasma proteins may act to decrease sensitivity to hormonal stimulation. Teleologically this could serve the function of allowing the specialized processes which are involved in wound repair to be regulated by local factors and to become transiently autonomous free from centralized control. This may explain the seeming priority of the wound in regard to nutrient usage. This hypothesis may also explain the increases in numerous plasma proteins, an acute phase like response, following exercise of duration and exertion sufficient to produce muscle tissue damage (32).

#### SUMMARY

In response to injury, the concentrations of several plasma proteins are characteristically altered. In part, these changes reflect an essential contribution of many of these proteins, acting in concert, to the processes involved in wound healing. There is evidence that plasma proteins support tissue repair by metabolic as well as functional activity. Specifically, plasma proteins may directly facilitate wound healing by: provision of carbohydrates, lipids and amino acids in a usable form as biosynthetic precursors and energetic substrates; the transport of trace metal cofactors involved in various wound repair processes; adhesion of regenerating tissue; modulation of the rate of structural protein synthesis; alignment of collagen subunits; organization of cellular elements associated with removal of damaged tissue and wound repair; prevention of autoimmune reactions; hormone transport and local modulation of hormonal effects; neutralization of the potentially toxic products of the inflammatory response and the inhibition of microbial invasion and colonization.



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